

SUMMARY

The invention relates to a method for optically detecting at least one entity which is arranged on and/or in a substrate (60) preferably being arranged on a support (61), said substrate (60) having a refraction-index which is different from the one of the at least one component adjacent to the substrate (60). The at least one entity is scanned with a measuring volume (70) using at least one radiation source (10) and a confocal optic (32) or an optic (32) made for multi-photon-excitation, thereby receiving measuring values of optical parameters which are processed by means of signal processing for characterization of the at least one entity. Said entity substantially maintains its position in respect to the substrate (60) or the support (61) for the duration of the recording. Before and/or during the scanning process an auxiliary focus (71) is generated by means of at least one radiation source (11) and an optic (34), said auxiliary focus (71) is at least partly arranged on the interface (62) between substrate (60) and adjacent component or on another interface (62) having a defined spacial relation to said entity. A retroreflection from the auxiliary focus is detected by at least one detector (21) and is used for measuring the position of the interface (62) and, thus, for indirectly positioning the measuring volume (70). In the method according to the invention, the position of the auxiliary focus (71) relative to the measuring volume (70) is adjustable in a defined manner. Thus, possible applications of the method as well as an apparatus for performing said method are described.

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